EXTRACTION OF ANTIOXIDANT COMPOUNDS FROM RED PITAYA USING SOXHLET EXTRACTION METHOD

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I declare that this thesis entitled "*Extraction of antioxidant compounds from red pitaya using Soxhlet extraction method*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Date : 20 APRIL 2009

Special Dedication of This Grateful Feeling to My…

Beloved father and mother;
Mr. Wan Abdul Jalil b Wan Idris and Mrs. Hashimah bt Yusuf

Loving brothers and sisters;
Wan Norsuzihana, Wan Norafadila, Wan Hasrul Faez and Wan Nur Syabihah

Supportive families;
Uncles and Aunties

For Their Love, Support and Best Wishes.

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ABSTRACT

The fruits of *Hylocereus cacti,* known as red pitaya, have recently drawn much attention of growers worldwide, not only because of their red-purple colour and economic value as food products, but also for their antioxidant compounds especially Vitamin C. Vitamin C is essential to a health diet as well as being a highly effective antioxidant that capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals, which start chain reactions that can damage cells. In this research, Soxhlet extractor was used to extract the Vitamin C from red pitaya. Then the extracts obtained were evaporated using rotary evaporator to get concentrated oil. The oil produced was analyzed using High Performance Liquid Chromatograph and Ultraviolet-Visible Spectrophotometer. The effects of different drying temperature, solvent polarity and solid to solvent ratio on extraction yields were investigated. The drying temperatures used were 35^0C, 50^0C, 60^0C and 70^0C. The solvents used were methanol, ethanol, hexane and toluene. In term of solid to solvents ratio, it was set at 5g: 400mL, 10g: 400mL, 15g: 400mL and 20g: 400mL. It was found that drying temperature at 60^0C with methanol as solvent and 15: 400mL solid to solvent ratio give the highest yields of vitamin C which was 3.87 mg/L and 61.67% of antioxidant activity. Thus, it can be concluded as the most suitable operating parameter for extraction of Vitamin C from red pitaya using Soxhlet extraction method.

ABSTRAK

Buah *Hylocereus cacti* atau lebih dikenali sebagai pitaya merah (buah naga) telah menarik perhatian para petani sedunia sejak kebelakangan ini, bukan sahaja kerana warnanya yang merah keunguan dan nilai ekonomi sebagai produk makanan, tetapi juga kerana kandungan antioksidanya terutama sekali Vitamin C. Vitamin C diperlukan dalam diet seimbang dan sebagai antioksida yang sangat efektif untuk melambatkan atau mencegah proses pengoksidaan molekul-molekul lain. Tindakbalas pengoksidaan menghasilkan radikal bebas yang boleh menimbulkan kerosakan terhadap sel-sel didalam badan. Dalam kajian ini, kaedah Soxhlet digunakan untuk mengekstrak kandungan Vitamin C dari buah pitaya merah. Hasil pengekstrakan kemudian diruapkan mengunakan *rotary evaporator* untuk mendapatkan minyak yang pekat. Seterusnya minyak terhasil diuji menggunakan *High Performance Liquid Chromatograph* dan *Ultraviolet-Visible Spectrophotometer*. Kesan terhadap suhu pengeringan, kekutuban pelarut, dan nisbah pepejal kepada pelarut telah dikaji. Suhu pengeringan yang digunakan ialah 35^0C, 50^0C, 60^0C dan 70^0C. Pelarut yang digunakan ialah metanol, etanol, heksana dan toluena. Nisbah pepejal kepada pelarut yang digunakan pula ialah 5g: 400mL, 10g: 400mL, 15g: 400mL dan 20g: 400mL. Kajian menunjukkan suhu pengeringan pada 60^0C, penggunaan metanol sebagai pelarut dan nisbah 15g pepejal kepada 400mL pelarut menghasilkan nilai vitamin C yang tertinggi iaitu 3.87mg/L dan 67.6% untuk antioksida aktiviti. Oleh itu, keadaan ini boleh disimpulkan sebagai parameter terbaik bagi proses pengekstrakan Vitamin C dari buah pitaya merah menggunakan kaedah Soxhlet.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Antioxidant is a molecule that capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that can damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols (en.wikipedia.org).

There are two or three major vitamin groups such as vitamin A, C and E that work in the body as antioxidants. Vitamin E and Vitamin C are very powerful natural antioxidants. Many vitamins are proven antioxidants, which mean they are able to fight against free radicals in the body. Vitamin C is a powerful antioxidant, and is also needed for the production of serotonin. Vitamin E also works synergistically with the mineral selenium that helps body to create other antioxidants. Although both vitamin A and vitamin C are good antioxidants vitamin E is the most effective and potential antioxidants for the health of your eyes (Miller, 1993).

Nowadays people prone to get synthetic antioxidant as their supplement or vitamins compared to natural antioxidant. Synthetic vitamins consist of synthetic chemicals. Only vitamins from dietary sources or edible plants can be considered nutrients for the body. Synthetic vitamins are like any other synthetic molecules but because of their antioxidant nature, they are able to donate one electron, which they do not remain stable but are broken down in a metabolic process. Consequently it yields hydrogen peroxide. Administering synthetic vitamins to persons with disease can thus be counter-productive. These people already have a problem associated with or directly caused by excess free radicals, including hydroxyl radicals. Adding substances into their biological system that can lead to the formation of more hydroxyl radicals only exacerbates their free radical biochemistry (www.oregonstate.edu). There are several studies that show that synthetic vitamins are harmful. The first study was at1994 where the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC) involving few men who were heavy smokers and alcohol drinkers. The volunteers were either given 20 milligrams of synthetic beta carotene, vitamin E, a combination of the two, or a placebo. The expected outcome suggested that there was an 18 percent increase in lung cancer rates in the beta carotene-only group. Cigarette smoke contains about 4000 toxic chemicals, of which 40 are known carcinogens. The metabolic breakdown of synthetic chemicals and alcohol yields toxic metabolites that yield the superoxide radical and hydrogen peroxide. Similarly, the breakdown of synthetic beta carotene and synthetic vitamin E also results in the same biochemical problem in the liver and that simply adds to creating excess free radicals and oxidative stress from hydroxyl radicals. Hydroxyl radicals can cause damage to membranes and DNA molecules and transform normal cells into cancer cells. In other words, synthetics accelerate free radical biochemistry.

According to these situations, some action needs to be taken out to avoid the side effect of the using synthetic antioxidants. One of the ways is by extracting the antioxidant from the fresh food that contains high level of antioxidants itself. Extraction is the best way to keep maintains the sufficient nutrients in the food. There are several type of extraction method such as Soxhlet extraction, hydro distillation, ultrasonic, and others. The general definition of extraction is the method of separation process by using a solvent in which the desired substance dissolves in while the undesired substance does not dissolve in. The Soxhlet extraction is the most conventional of all methods and consists of a simple distillation process repeated a number of times. Soxhlet extraction method is straightforward and inexpensive. The sample phase is always in contact with fresh solvent, thereby enhancing the displacement of target compound from the matrix and the compound are not discomposed due to the moderate extraction condition.

In this research, works will be done to extract the antioxidant, specifically Vitamin C from red dragon fruit or also known as red pitaya. Red pitaya is a great healthy fruit to eat with lots of beneficial nutrients which are readily metabolized from the natural red pitaya fruit. Vitamin C in red pitaya is more easily absorbed than Vitamin C from a pill supplement. Lots of Vitamin C is in red pitaya. The amount of Vitamin C in red pitaya dried is 10 times more than amount of Vitamin C in fresh red pitaya.

Besides, red pitaya can helps to reduce levels of glucose in blood especially for diabetes patient. Other than that, the nutrient profile contained in red pitaya have full of dietary fiber, Vitamin C and even have a high level of antioxidant. These will enhance the nutritional value to our body particularly from the dried or fresh red pitaya fruit.

1.2 Identification of Problem

Everyday, our body easily exposed to mutated and oxidized cells, known as "free radicals". With a strong immune system, the human body can recognize and get rid of these cells. Cells can become mutated or oxidized naturally as they are replicating in the body, or from stress, smoking, age, and sun damage.

Our body has developed a natural way to rid off of these cells through the consumption and the use of natural antioxidants. The body naturally creates some antioxidants, but it relies heavily on a proper diet to get the rest of its natural antioxidants. Free radicals are the forerunners of devastating diseases like cancer, osteoporosis and heart disease. Antioxidants also can help rid off these free radicals from our body (http://oregonstate.edu).

From the early discovery based on red pitaya, it is worth to extract the antioxidant from dragon fruit that is very useful for human health in term of avoiding the side effect of using synthetic antioxidants. In addition, antioxidants have many industrial uses, such as preservatives in food and cosmetics and also preventing the degradation of rubber and gasoline. With high antioxidant compound and the feasible market price; that is why dragon fruit is the choosen material for this research. However this research is just a preliminary step before it become commercialize.

1.3 Statement of Objective

The main objective of this research is to extract the Vitamin C from red pitaya using Soxhlet extraction method.

1.4 Scope of Study

In order to achieve the objectives, there are few scopes of work that have been identified as follows:

i. Study the effect of different drying temperature on extraction yield using oven at temperature 35^0C, 50^0C, 60^0C and 70 ^{0}C.
ii. Investigate the effect of different solvents using methanol, ethanol, n-hexane and toluene on extraction yields.
iii. Examine the effect of different solid to solvent ratios which are 5g:400 mL, 10g:400 mL, 15g:400 mL and 20g:400 mL to extraction yields
iv. Establish the suitable operating parameter for extraction of Vitamin C from red pitaya using Soxhlet extraction.

v. Analyze the extraction yields by using high performance liquid chromatography (HPLC) and UV-Vis.

1.5 Rational and Significant

Currently red pitaya become popular since people know about the nutritious values inside the fruit. With high level of antioxidant content and lower price in market, this fruit is potential as a good alternative for natural antioxidant. This will expand the usage of red pitaya and also can reduce the cost of Vitamin C production in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Fruit Material

Dragon fruit (Hylocereus undatus (Haw.)) or pitaya, have been grown in Vietnam for at least 100 years, following their introduction by the French (Mizrahi *et al.*, 1997). Locally the fruit is known and sold as Thanh Long, or ‘Green Dragon’, a description associated with the green colour of the immature fruit, and the ‘dragon-like’ appearance of the ‘scales’ or bracts on the fruit surface. Native to Mexico and Central and South America, the vine-like epiphytic Hylocereus cacti are also cultivated in Southeast Asian countries such as Taiwan, Vietnam, the Philippines, and Malaysia. They are also found in Okinawa, Israel, northern Australia and southern China. Hylocereus blooms only at night; they have large white fragrant flowers of the typical cactusflower shape that are often called Moonflower or Queen of the Night. Sweet pitayas have a creamy pulp and a delicate aroma (en.wikipedia.org).

Sweet pitayas come in three types, all with leathery, slightly leafy skin:

i. *Hylocereus undatus* (Red Pitaya) has red-skinned fruit with white flesh. This is the most commonly-seen "dragonfruit".
ii. *Hylocereus costaricensis* (Costa Rica Pitaya, sometimes called *H. polyrhizus*) has red-skinned fruit with red flesh

iii. Hylocereus megalanthus (Yellow Pitaya) has yellow-skinned fruit with white flesh

The fruit weighted is from 150-600 grams and the flesh, which is eaten raw, is mildly sweet and low in calories. Eating the fruit is sometimes likened to that of the kiwifruit due to a prevalence of sesame seed-sized black crunchy seeds found in the flesh of both fruits which make for a similar texture upon consumption. The skin is not eaten. The fruit may be converted into juice or wine; the flowers can be eaten or steeped as tea. Although the tiny pitaya seeds are eaten with the flesh, the seeds are indigestible. It is generally recommended that dragon fruit be eaten chilled, for improved flavor. Ingestion of significant amounts of red dragon fruit may result in a reddish discoloration of the urine (www.medicine.com.my). Figure 2.1 shows flesh of Costa Rica Pitayas and Figure 2.2 shows Red Pitaya (Hylocereus undatus).

Figure 2.1: Flesh of Costa Rica Pitayas

Figure 2.2: Red Pitaya (*Hylocereus undatus*)

2.1.2 Cultivation

Red pitaya has adapted to live in dry tropical climates with a moderate amount of rain. The dragon fruit sets on the cactus-like trees 30-50 days after flowering and can sometimes have 5-6 cycles of harvests per year. There are some farms in Vietnam that produce 30 tons of fruit per hectare every year (Jacobs, 1999).

Overwatering or excessive rainfall can cause the flowers to drop and fruit to rot. Birds can be a nuisance. The bacterium Xanthomonas campestris causes the stems to rot. Dothiorella fungi can cause brown spots on the fruit, but this is not common (Felger and Moser, 1985)

2.1.3 Nutritional Information

Red-skinned pitayas are rich in vitamins, especially Vitamin C. Pitayas are rich in fiber and minerals, notably phosphorus and calcium. Red pitayas are richer in the former, yellow ones in the latter. In Taiwan, diabetics use the fruit as a food substitute for rice and as a source of dietary fibre. Pitayas are also rich in phytoalbumins which are highly valued for their antioxidant properties. Costa Rica Pitayas are rich in antioxidants which prevent the formation of cancer-causing free radicals (Jacobs *et al.*, 1999). The typical nutritional value per 100g of raw pitaya (of which 55 g are edible) is shown in Table 2.1.

Table 2.1: Typical nutritional value per 100g of raw pitaya (Ariffin, 2008)

Nutritious	**Weight**
Water	80-90g
Carbohydrates	9-14g
Protein	0.2-0.5g
Fat	0.1-0.6g
Fiber	0.3-0.9g
Ash	0.4-0.7g
Calories	35-50g
Calcium	6-10g
Iron	0.3-0.7mg
Niacin (Vitamin B_3)	0.2-0.45mg
Ascorbic Acid (Vitamin C)	4-25mg
Carotene (Vitamin A)	traces
Riboflavin (Vitamin B_2)	traces

2.2 Antioxidant

2.2.1 Definition

Antioxidant is a molecule that capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols (en.wikipedia.org).

2.2.2 The Importance of Antioxidant

The leading causes of death in the United States are cardiovascular diseases and cancers. Similarly, in Taiwan, around 27% of deaths are from cancer and 18% of deaths are from cardiovascular and heart diseases (Department of Health Web, 2004). It was estimated by Willet (1994) that roughly 32% (range of 20%–42%) of deaths from cancer could be avoided by dietary modification.

Epidemiological studies have strongly suggested that diet plays an important role in the prevention of chronic diseases (Bauman *et al.,* 2004; Willet, 1995). Polyphenolics, thiols, carotenoids, tocopherols, and glucosinolates commonly found in fruits, vegetables and grains, provide chemoprotective effects to combat oxidative stress in the body and maintain balance between oxidants and antioxidants to improve human health (Adom and Liu, 2002; Dragsted *et al.*, 1993; Jia *et al.*, 1999; Wolfe *et al.*, 2003). An imbalance caused by excess oxidants leads to oxidative stress, resulting in damage to DNA and protein and increased risk of degenerative diseases such as cancer (Farombi *et al.,* 2004).

Consumption of fresh fruits and vegetables, as well as grains, has been associated with reduced risk of coronary heart disease (CHD) (Bazzano *et al.,* 2003; Joshipura *et al*., 2001; Srinath Reddy and Katan, 2004), stroke (Gillman *et al*., 1995; Voko *et al.,* 2003), symptoms of chronic obstructive pulmonary disease (Fabricius & Lange, 2003; Liu *et al.,* 2004), and different types of cancer, including breast and ovarian cancer (Duncan *et al.,* 2004) and colon cancer (Frydoonfar *et al.,* 2003). Polyphenolic compounds, widely distributed in higher plants, have been found to have potential health benefits that are believed to arise mainly from their antioxidant activity (Liu, 2003). There is considerable scientific and public interest in the important role that antioxidants may play in health care, such as by acting as cancer chemopreventive and anti-inflammatory agents and by reducing risk of cardiovascular mortality (Cos *et al*., 2004).

Antioxidants are substances that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species, which include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non- radicals such as hydrogen peroxide, hypochlorous, etc. They scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi *et al.,* 2001). The most abundant antioxidants in fruits are polyphenols and Vitamin C, Vitamins A, B and E and carotenoids are present to a lesser extent in some fruits. These polyphenols, most of which are flavonoids, are present mainly in ester and glycoside forms (Fleuriet and Macheix, 2003).

2.2.3 Antioxidant in Dragon Fruit

The fruits of *Hylocereus cacti*, known as red pitaya or pitahaya, have recently drawn much attention of growers worldwide, not only because of their red-purple colour and economic value as food products, but also for their antioxidative activity from the betacyanin contents (Wybraniec and Mizrahi, 2002).

In cacti, the most important fruit pigments are the betacyanins and betaxanthins (Wybraniec *et al.,* 2001). Betalains, composed of red-violet betacyanin and yellow betaxanthins, are water-soluble pigments that provide colours in flowers and fruits. The known betacyanin pigments of Hylocereus polyrhizus flesh are betanin, phyllocactin (60-O-malonylbetanin), and a recently discovered betacyanin, hylocerenin (5-O-[60-O-(300-hydroxyl- 300-methyl-glutaryl)-b-D-glucopyranoside) (Wybraniec and Mizrahi, 2002; Wybraniec *et al*., 2001). Betacyanin, with 5-O-glycosides (betanin) or 6-O-glycosides, is commonly detected in plants. More-complicated esterification of 5-O-glycosides with hydrocinnamic acids such as ferulic or p-coumaric acids (Strack *et al.,* 1993) and malonic acid (Kobayashi *et al.,* 2000; Minale *et al.,* 1996) have also been studied.

It has been reported that betalains from Amaranthus (Cai *et al.*, 2003) and beet roots (Escribano *et al.*, 1998) demonstrated antiradical and antioxidant activity. Betanin, from red beet, effectively inhibited lipid peroxidation and heme decomposition, suggesting that these pigments may provide protection against certain oxidative stress-related disorders (Kanner, *et al.*, 2001). Polyphenolics, on the other hand, play an important role in antioxidant activity. They have evidently shown antiproliferative activity or cytotoxicity to human oral cancer cells (Seeram, Adams, Hardy, & Heber, 2004), melanoma cells (Rodriguez et al., 2002), and lung metastasis induced by B16F10 melanoma cells (Menon and Kuttan, 1995).

2.3 Soxhlet Extraction Process

2.3.1 Soxhlex Extractor

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet (Dingler's *et al*., 1879). It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has only a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Figure 2.3 shows example of Soxhlet extractor.

According to the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent, usually hexane or petroleum ether, under reflux in a special glassware.